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chromosomes. Exemplary repeated nucleotide sequences include (1) SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 51 or SEQ ID NO: 52, or fragments or variants thereof, (2) combinations of any of these *Brassica* sequences or a fragment or variant thereof with another *Brassica*-derived centromeric nucleotide sequence, (3) combinations of any of these *Brassica* sequences or a fragment or variant thereof with a centromeric nucleotide sequence derived from a different plant species, and (4) combinations of any of the above with artificially synthesized centromeric nucleotide sequences.

In exemplary embodiments the invention also contemplates mini-chromosomes or other vectors comprising a repeated nucleotide sequence derived from a *Glycine max* plant and adchromosomal plants or parts containing these mini-chromosomes. Exemplary repeated nucleotide sequences include (1) SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO:26, or fragments or variants thereof, (2) combinations of any of these soybean sequences or a fragment or variant thereof with another soybean-derived centromeric nucleotide sequence, (3) combinations of any of these soybean sequences or a fragment or variant thereof with a centromeric nucleotide sequence derived from a different plant species, and (4) combinations of any of the above with artificially synthesized centromeric nucleotide sequences.

In exemplary embodiments, the invention also contemplates mini-chromosomes or other vectors comprising fragments or variants of the genomic DNA inserts of the BAC clones [identified as BB5, SB6, TB99, ZB19, or ZB113] deposited on February 23, 2005 with the American Type Culture Collection (ATCC), P.O. Box 1549 Manassas, VA 20108, USA , under Accession Nos. PTA-6601, PTA-6602, PTA-6603, PTA-6604 and PTA-6605, respectively, or naturally occurring descendants thereof, that retain the ability to segregate during mitotic or meiotic division as described herein, as well as adchromosomal plants or parts containing these mini-chromosomes. Other exemplary embodiments include fragments or variants of the genomic DNA inserts of any of the BAC clones identified herein, or descendants thereof, and fragments or variants of the centromeric nucleic acid inserts of any of the vectors or mini-chromosomes identified herein.

In other exemplary embodiments, the invention contemplates mini-chromosomes or other vectors comprising centromeric nucleotide sequence that when hybridized to 1, 2, 3, 4, 5, 6, 7, 8 or more of the probes described in the examples

In another example, individual satellite repeats from soybean BAC clone SB12R2-3 (SEQ ID NO: 24) showed an average of 91.3% (s.d.=11.3%) identity to each other, with specific regions showing significantly higher and lower levels of variability. Comparing the satellite repeat consensus from SB12R2-3 to that obtained from randomly sampled soybean satellite sequences ChrGm1 (SEQ ID NO: 25) and ChrGm2 (SEQ ID NO: 26), see U.S. Patent Application 20030124561: *Plant centromere compositions*) identified several bases that differed significantly (χ^2 test, $P < 0.05$). The SB12MC satellite repeats showed an average length of 91.07 ± 0.40 bp, similar to the ChrGm2 91-base consensus and differing from the ChrGm1 92-base consensus. An alignment of the of consensus centromere satellite repeats is set out in Figure 6.

Exemplary embodiments of centromere nucleic acid sequences according to the present invention include fragments or variants of the genomic DNA inserts of the BAC clones [identified as BB5, SB6, TB99, ZB19, or ZB113 deposited on February 23, 2005 with the American Type Culture Collection (ATCC), P.O. Box 1549 Manassas, VA 20108, USA , under Accession Nos. PTA-6601, PTA-6602, PTA-6603, PTA-6604 and PTA-6605, respectively, that retain the ability to segregate during mitotic or meiotic division as described herein. Variants of such sequences include artificially produced modifications as described herein and modifications produced via passaging through one or more bacterial, plant or other host cells as described herein.

Vectors comprising one, two, three, four, five, six, seven, eight , nine, ten, 15 or 20 or more of the elements contained in any of the exemplary vectors described in the examples below are also contemplated.

The invention specifically contemplates the alternative use of fragments or variants (mutants) of any of the nucleic acids described herein that retain the desired activity, including nucleic acids that function as centromeres, nucleic acids that function as promoters or other regulatory control sequences, or exogenous nucleic acids. Variants may have one or more additions, substitutions or deletions of nucleotides within the original nucleotide sequence. Variants include nucleic acid sequences that are at least 50%, 55%, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to the original nucleic acid sequence. Variants also include nucleic acid sequences that hybridize under low, medium, high or very high stringency conditions to the original nucleic acid sequence.

BB60	D	Hi CANREP, Meth (Hpa)	3. Complex ladder
BB63	D	Hi CANREP, Meth (Hpa)	2. Simple ladder
BB70	I	Hi CANREP, Moderate Meth	2. Simple ladder
BB71	E	Hi CANREP, Meth (Sau)	3. Complex ladder
BB76	I	Hi CANREP, Moderate Meth	1. Complex
BB104	I	Hi CANREP, Moderate Meth	n/d*

n/d*: Gel too faint to score

B. oleraceae (broccoli) BAC BB5 was deposited with the American Type Culture Collection (ATCC) P.O. Box 1549 Manassas, VA 20108, USA on February 23, 2005 and assigned Accession No. PT-6601.

To determine the molecular weight of centromere fragments in the BAC libraries, a frozen sample of bacteria harboring a BAC clone was grown in selective liquid media and the BAC DNA harvested using a standard alkaline lysis method. The recovered BAC DNA was restriction digested and resolved on an agarose gel. Centromere fragment size was determined by comparing to a molecular weight standard.

Cre/lox recombined donor DNA and BAC centromere DNA was delivered into bacteria and plated on selective solid media. To determine the molecular weight of centromere fragments in retrofitted mini-chromosomes, three bacterial colonies harboring a mini-chromosome were independently grown in selective liquid media and the BAC DNA harvested using a standard alkaline lysis method. The recovered BAC DNA was restriction digested and resolved on an agarose gel. Centromere fragment size was determined by comparing to a molecular weight standard. If variation in centromere size was noted, the mini-chromosome with the largest centromere insert was used for further experimentation.

Example 2
Assembly and Components of *Brassica* Mini-chromosomes

Two methods have been developed to construct plant mini-chromosomes. The first method relies on cre/lox recombination in which a bacterial mini-chromosome (BAC) vector containing plant centromeric DNA and a loxP recombination site is recombined, by the action of cre recombinase, with a donor vector carrying plant gene expression cassettes to generate a plant mini-chromosome. The second method uses restriction enzyme digestion and ligation to produce two

Class	Class Properties	Probe Hybridization Range				TEL	# clones identified
		LESAT C2	LESAT E1	LEGATA REP E12	Hpa II (METH)		
G	High E1 and TEL	N/A	>=8	N/A	N/A	>=8	8
H	High E1 only	N/A	>=8	<=4	<=6	N/A	89
I	High TEL only	N/A	<=4	N/A	<=4	>=8	49
J	High Meth only	N/A	N/A	<=4	>=7	<=4	15
K	High E12 only	N/A	N/A	>=7	<=4	<=4	2
Total **							451

* Values represent hybridization intensities of an individual BAC to each probe on a scale of 1 to 10. Values were normalized
N/A = not applicable

A number of representative clones from each class were chosen to yield a total of 278 BAC clones for further analysis by restriction digest fingerprinting. The BAC clones were fingerprinted (Table 26) based on restriction sites found in the centromere specific sequence(s) as described in Example 1. The restriction enzyme *HinfI* was used to digest the BAC clones. After fingerprinting, 100 BACs were selected for further testing using the method described in Example 1.

L. esculentum (tomato) BAC TB99 was deposited with the American Type Culture Collection (ATCC) P.O. Box 1549 Manassas, VA 20108, USA on February 23, 2005 and assigned Accession No. PTA-6603.

Thirty BAC clones (from the original 278) were selected for mini-chromosome construction based on the fingerprint class which was defined as a simple or complex laddering pattern. Table 26 lists the fingerprint patterns for a selected set of 26 Tomato BAC clones. Tomato fingerprints were classified into 3 classes: 1. high complexity (multiple large bands with no indication of laddering), 2. low ladder (predominant bands at multiples of the unit repeat size for the centromere satellite, and 3. complex ladder (features of both previous types). Table 27 lists the fingerprint classes for 7 selected tomato BACs. The preferred BACS have an *. Table 27 lists the fingerprint classes for 11 selected *Brassica* BACs.

Table 35: Restriction endonuclease fingerprint classification for 10 selected soybean BACs

			Fingerprint Class	
BAC Number	**Class**	**Class Properties**	HinfI	DdeI
SB3	H	High TRS and 3X1	3. complex ladder	3. complex ladder
SB6	B	High TRS	2. simple ladder	2. simple ladder
SB9	H	High TRS and 3X1	3. complex ladder	2. simple ladder
SB11	B	High TRS	3. complex ladder	3. complex ladder
SB12	B	High TRS	3. complex ladder	n/d*
SB22	A/L	High 3X1/RE	2. simple ladder	1. complex
SB38	H	High TRS and 3X1	n/d*	3. complex ladder
SB50	B	High TRS	3. Complex ladder	n/d*
SB116	A	High 3X1	2. simple ladder	n/d*
SB125	B	High TRS	3. complex ladder	n/d*

G. Max (soybean) BAC SB6 was deposited with the American Type Culture Collection (ATCC) on P.O. Box 1549 Manassas, VA 20108, USA on February 23, 2005 and was assigned Accession No.PTA-6602.

Construction of Mini-chromosome

Each of the soybean BAC clones identified above were constructed using a Cre-Lox Recombination-Donor as described in Example 2. Soybean mini-chromosomes were constructed from a total of 33 BACs using donor vector pCHR151 in this assembly process, and were subsequently tested in several different soybean cell lines. Mini-chromosome genetic elements within the pCHR151 are described above in Table 10. The Soybean mini-chromosomes were used to transform broccoli plants (see Table 37 below).

Identification of functional soybean centromeres

Functional testing of mini-chromosomes using transient assays as described may be carried out as in Example 3. Mini-chromosomes are delivered to the soybean cells using wet biolistic as described in Example 2. After DNA delivery, the cell population is then monitored for fluorescent protein expression over the course of one to several weeks. Mini-chromosomes containing active centromeres

strong hybridization to Cent4. Class VII (HiTr1 LoHet) BAC clones had strong hybridization toTR-1 but low hybridization to MZEHETRO. Class VIII (LoTr1 HiHet) BAC clones had strong hybridization to MZEHETRO but low hybridization to TR-1. Class IX (HiTr1 HiHet) BAC clones had strong hybridization to both TR-1 and MZEHETRO.

A number of representative clones from each class were chosen to yield a total of 315 BAC clones for further analysis by restriction digest fingerprinting.

The 315 BAC clones were fingerprinted based on restriction sites found in the centromere specific sequence(s). Fingerprinting was used to evaluate the sequence composition of the large numbers of BAC clones and to compare their similarity to each other by comparing the restriction enzyme digest fragment patterns. A sequence with a tandem repeated sequence will show a single intense band of unit repeat size when digested with a restriction enzyme that cuts within the unit repeat. Second, BAC clones with similar sequences will show similar patterns of restriction fragments in a digest.

BAC DNA was extracted from bacteria using methods familiar to those in the art. Restriction enzymes *Hpa*II and *Msp*I were used to digest BAC clones in Classes I through VI, and restriction enzyme *NdeI* was used to digest BAC clones in classes VII through IX.

Z. mays (corn) BACs ZB19 and ZB113 were deposited with the American Type Culture Collection (ATCC) P.O. Box 1549 Manassas, VA 20108, USA on February 23, 2005 and assigned Accession Nos. PTA-6604 and PTA-6605, respectively.

Example 15
Construction of Maize Mini-chromosomes

The 115 BAC clones identified in Example 1 were grown up and DNA was extracted for mini-chromosome construction using NucleoBond™ Purification Kit (Clontech). To determine the molecular weight of centromere fragments in the BAC libraries, a frozen sample of bacteria harboring a BAC clone was grown in selective liquid media and the BAC DNA harvested using a standard alkaline lysis method. The recovered BAC DNA was restriction digested and resolved on an agarose gel. Centromere fragment size was determined by comparing to a molecular weight standard.